

We claim

1. A recombinant adeno-associated virus (AAV) virion comprising a nucleic acid molecule, said nucleic acid molecule comprising a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE.
2. The recombinant AAV virion of claim 1, wherein the transcriptional promoter region comprises at least five EcREs.
3. The recombinant AAV virion of claim 1, wherein the promoter is a heat shock protein (Hsp) promoter sequence.
4. The recombinant AAV virion of claim 1, wherein the transcriptional promoter region further comprises at least one enhancer sequence.
5. The recombinant AAV virion of claim 4, wherein the enhancer sequence is an SP1 enhancer sequence.
6. The recombinant AAV virion of claim 4, wherein the transcriptional promoter region comprises three SP1 enhancer sequences.
7. A recombinant adeno-associated virus (AAV) virion comprising a nucleic acid molecule, said nucleic acid molecule comprising a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises five ecdysone-responsive elements positioned upstream of a heat shock protein (Hsp) promoter sequence, wherein the transcriptional promoter region is capable

of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell.

8. The recombinant AAV virion of claim 7, wherein the transcriptional promoter  
5 region further comprises at least one enhancer sequence.

9. The recombinant AAV virion of claim 8, wherein the enhancer sequence is an  
SP1 enhancer sequence.

10. The recombinant AAV virion of claim 8, wherein the transcriptional promoter  
10 region comprises three SP1 enhancer sequences.

11. A recombinant adeno-associated virus (AAV) virion comprising a nucleic  
acid molecule, said nucleic acid molecule comprising a first coding sequence encoding an  
15 ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription of said first coding sequence in a mammalian cell.

12. The recombinant AAV virion of claim 11, wherein the nucleic acid molecule  
further comprises a second coding sequence encoding a retinoid-X-receptor (RXR)  
20 operably linked to control elements capable of directing the *in vivo* transcription of said  
second coding sequence in a mammalian cell.

13. The recombinant AAV virion of claim 12, wherein said first coding sequence  
and said second coding sequence are present in the same expression cassette.  
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14. The recombinant AAV virion of claim 12, wherein said first coding sequence  
and said second coding sequence are present in separate expression cassettes.

15. A recombinant adeno-associated virus (AAV) virion comprising a nucleic  
30 acid molecule, said nucleic acid molecule comprising first and second coding sequences,

wherein said first coding sequence encodes an ecdysone receptor (EcR) and said second coding sequence encodes a retinoid-X-receptor (RXR), wherein said first and second coding sequences are operably linked to control elements capable of directing the *in vivo* transcription thereof in a mammalian cell.

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16. The recombinant AAV virion of claim 15, wherein said first coding sequence and said second coding sequence are present in the same expression cassette.

10 17. The recombinant AAV virion of claim 15, wherein said first coding sequence and said second coding sequence are present in separate expression cassettes.

15 18. A recombinant adeno-associated virus (AAV) virion comprising a nucleic acid molecule, said AAV vector comprising a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said coding sequence in a mammalian cell.

19. A method of producing recombinant adeno-associated virus (AAV) virions comprising:

20 (a) transfecting a host cell with (i) an AAV vector comprising a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; (ii) AAV helper functions; and (iii) AAV accessory functions,

25 wherein said transfecting is done under conditions that allow the formation of recombinant AAV virions; and

(b) purifying the recombinant AAV virions from the host cell.

30 20. A method of producing recombinant adeno-associated virus (AAV) virions comprising:

5 (a) transfecting a host cell with (i) an AAV vector comprising a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises five ecdysone-responsive elements positioned upstream of a heat shock protein (Hsp) promoter sequence, wherein the transcriptional promoter region is capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell; (ii) AAV helper functions; and (iii) AAV accessory functions,

10 wherein said transfecting is done under conditions that allow the formation of recombinant AAV virions; and  
(b) purifying the recombinant AAV virions from the host cell.

21. A method of producing recombinant adeno-associated virus (AAV) virions comprising:

15 (a) transfecting a host cell with (i) an AAV vector comprising a first coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription of said first coding sequence in a mammalian cell; (ii) AAV helper functions; and (iii) AAV accessory functions,

wherein said transfecting is done under conditions that allow the formation of recombinant AAV virions; and

20 (b) purifying the recombinant AAV virions from the host cell.

22. The method of claim 21, wherein the AAV vector further comprises a second coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said second coding sequence in a  
25 mammalian cell.

23. A method of producing recombinant adeno-associated virus (AAV) virions comprising:

30 (a) transfecting a host cell with (i) an AAV vector comprising a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of

directing the *in vivo* transcription of said coding sequence in a mammalian cell; (ii) AAV helper functions; and (iii) AAV accessory functions,

wherein said transfecting is done under conditions that allow the formation of recombinant AAV virions; and

- 5 (b) purifying the recombinant AAV virions from the host cell.

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24. A method of inducing gene expression in a mammalian cell, said method comprising:

- 10 (a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; and (ii) a second
- 15 recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription of said EcR coding sequence in a mammalian cell; and
- 20 (b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

- 25 25. The method of claim 24, wherein step (a) further comprises transducing the mammalian cell with (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said RXR coding sequence in the mammalian cell.

26. A method of inducing gene expression in a mammalian cell, said method comprising:

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- 5 (a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises five ecdysone-responsive elements positioned upstream of a heat shock protein (Hsp) promoter sequence, wherein the transcriptional promoter region is capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription of said EcR coding sequence in a mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription of said RXR coding sequence in the mammalian cell; and
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- 15 (b) providing ponasterone A to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

- 20 27. The method of claim 26, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.
28. The method of claim 27, wherein the enhancer sequence is an SP1 enhancer sequence.
- 25 29. The method of claim 27, wherein the transcriptional promoter region comprises three SP1 enhancer sequences.

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